STUDIES ON SYNTHETIC MEDIUMS

III. SOME ANIMAL EXPERIMENTS WITH ORGANISMS GROWN ON SYNTHETIC MEDIUM

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A number of organisms were found that grew on a synthetic medium of the following composition:

129.5	c c of molar	H_3PO_4
18.8	c c of molar	CH_3COOH
17.8	c c of molar	NH_4OH
100	c c of molar	NaOH
100	c c of molar	KOH
10	c c of 0.01%	Fe_2Cl_6
10	c c of 0.01%	$MgSO_4$
10	c c of 0.01%	$CaCl_2$

} diluted up to give one liter of medium

The medium, a water clear liquid having a hydrogen-ion concentration of 10^{-7N}, was tubed, and sterilized in steam on 3 successive days for 15 minutes. The organisms that grew in this medium did not change in their cultural and biochemical reactions after periods of growth varying from 1 day to 1 month. The object of this investigation was to find out if some of these organisms which were culturally unchanged, had changed in their behavior toward animals.

The organisms chosen were B. mucosus (Friedländer bacillus), Microspira cholerae, and B. typhosus (Hopkins' strain). The first two were tried for virulence and the typhoid for the production of antibodies.

A culture of B. mucosus was used which had grown in the synthetic medium for a period of 19 days, having been transplanted every 4 days, by transferring 0.1 c c of the old culture in a sterile pipet, to a new tube of synthetic medium. A transplant was made from this culture on agar and after 24 hours was washed off with sterile salt solution, and 0.5 c c of this, representing 0.05 of an agar slant, was injected intraperitoneally into a mouse at 11 a. m.; and the mouse, though still alive at 5 p. m., was found dead at 9 the next morning. The culture was recovered from the peritoneum and heart, the organisms having all the characteristics of the original culture, possessing a more marked capsule than the original culture.

Two other mice were inoculated with this culture—one directly from the synthetic culture. One mouse was given 1 c c of a synthetic culture, which had been transplanted several times, at 4 p. m. At the same time, another

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mouse was inoculated with some of the centrifugate of the same culture, washed and resuspended in salt solution to the original volume. One c c of this was injected intraperitoneally. Both mice were found dead at 9 the next morning, and as before, the organism was recovered from the peritoneal cavity and from the heart.

Of the culture of Microspira cholerae used, 0.05 of an agar slant would kill a rabbit weighing 1,885 gm. in less than 18 hours. The culture was then grown in the synthetic medium for several transplants, 4 days apart, and then transplanted to agar. This agar slant was emulsified in sterile salt solution and injected intravenously in a rabbit. This was done in three rabbits without any effect.

The results given by the two organisms would lead us to suppose that B. mucosus capsulatus had not changed in virulence while the Microspira cholorae had. Just why this should be so, is not apparent at present, but future work may throw light on the subject. Whether the presence of the capsule on B. mucosus is instrumental in preserving its virulence is a matter of conjecture.

TABLE 1

DETAILS OF METHODS EMPLOYED IN IMMUNIZING THE RABBITS

Rabbit Number	Weight in Gm.	Number of Injection	Amount of Injections, Slant	Date of Injections	Culture
50	1,875	1	0.02	5/ 4/18	Original
	2,020	2	0.1	5/ 9/18	Original
	1,950	3	0.1	5/13/18	Original
	1,905	4	0.1	5/18/18	Original
	1,885	5	0.2	5/23/18	Original
97	1,610	1	0.02	4/31/18	Synthetic
	1,699	2	0.10	5/ 4/18	Synthetic
	1,660	3	0.10	5/ 9/18	Synthetic
	1,630	4	0.10	5/15/18	Synthetic
	1,690	5	0.5	5/18/18	Synthetic
36	2,460	1	0.02	4/31/18	Synthetic
	2,270	2	0.05	5/ 4/18	Synthetic
	2,380	3	0.10	5/ 9/18	Synthetic
	2,170	4	0.10	5/15/18	Synthetic
	2,210	5	0.5	5/18/18	Synthetic

The experiments with the typhoid bacillus were for the purpose of determining whether or not agglutinins were produced by the organism after it had grown on the synthetic medium, and if so, whether the serum produced would agglutinate the original organism and whether a serum produced by the original organism would agglutinate the organism grown in the synthetic medium.

Three rabbits were immunized, two with an organism grown in the synthetic for a week and kept on the synthetic medium until used, being transferred to agar 24 hours before the injection was to be given. The third one was immunized with the original culture.

One week after the last injection, the rabbits were bled from the ear in order to procure just enough blood to test the titer of the serum with the result outlined in Table 2.

TABLE 2

RESULTS OBTAINED IN TESTING THE TITER OF THE SERUM

Rabbit No.	Culture	Dilutions of Serum										Control
		1:20	1:500	1:2,000	1:8,000	1:40,000	1:60,000	1:80,000	1:100,000	1:120,000	1:130,000	
36	Orig.	+++	+++	++	++	+	+	+	+	±	—	—
36	Synth.	+++	+++	++	++	+	+	+	+	±	—	—
50	Orig.	+++	++	++	+	+	+	+	—	—	—	—
50	Synth.	+++	++	++	++	+	+	+	—	—	—	—
97	Orig.	+++	+++	++	++	+	+	+	+	—	—	—
97	Synth.	+++	+++	++	++	+	+	+	+	—	—	—

That the rabbits should have developed serums of such a high titer is undoubtedly due in part to the fact that the organism was injected without killing, but must be due largely to the fact that the rabbits themselves possessed a marked resistance to typhoid bacilli, as I have immunized rabbits before with much larger doses of live typhoid and did not get serum of such high titer. The table shows that the serums produced by immunizing the rabbit with the original typhoid possesses as marked agglutinating powers for the synthetic organism as it does for the original with which it was immunized, while the reverse is true, the serum produced with the synthetic organism agglutinates the orginal just as well.

The only conclusion that we can draw is that the synthetic medium is suitable for growth of B. mucosus, not changing its biologic or cultural characteristics or its pathogenicity, while it apparently lacks something necessary to produce the substance responsible for the pathogenicity of Microspira cholerae. It might be possible to modify the medium slightly and produce the substance. However, it may be of advantage to be able to attenuate cholera in this manner. B. typhosus seems to find in medium all the essentials for its metabolism, remaining at the end of a month's growth on the synthetic culturally, biologically and immunologically the same.